Supplemental Figure S1. Segmental myocardial thickness following doxorubicin treatment. (A, B) After 24 h of doxorubicin treatment (4 dpf), the difference in myocardial thickness between end-systole and end-diastole was significantly decreased compared to control. (C, D) Changes in myocardial thickness recovered in most segments 48 h after the end of chemotherapy exposure (6 dpf). Two-sided t tests, * $P<0.05$, ** $P<0.01$, n=6-8 per group.
Supplemental Figure S2. Segmental comparison of Notch activation normalized to myocardial volume. (A-F) The Tg(cmlc2:mCherry;Tp1:gfp) line was imaged with confocal microscopy at 4 dpf. Notch activation was demonstrated by the green Tp1-gfp signal and the myocardium was delineated by the red cmlc2-mCherry signal. (G) Quantitative analysis of Tp1-gfp signal volume normalized to the segmental myocardium volume. Following 24-hour doxorubicin treatment, a robust increase in TP1 signal indicated enhanced Notch activity predominantly in segments IV – VI. Signals emanating from the atrioventricular valve were excluded from analyses. Two-sided t tests, * P<0.05, ** P<0.01, n=6-8 per group. Scale bar: 25 μm.
Supplemental Figure S3. **Segmental comparison of cardiomyocyte proliferation following chemo-induced injury.** Representative confocal microscopy images at 5 dpf, localizing proliferating cardiomyocytes (cmlc2:Venus-hGeminin, green) in the 3-D cardiac contour (cmlc2:mCherry, red) under control (A) and doxorubicin (B) conditions. (C) Quantitative analysis of segmental cardiomyocyte proliferation demonstrated a significant increase in segments II – VI following doxorubicin treatment. Two-sided t tests, * P<0.05, ** P<0.01, n=6-8 per group. Scale bar: 25 μm.
Supplemental Figure S4. The 3-D cardiac trabecular architecture at 7 dpf in response to doxorubicin induced injury and genetic manipulation. The restoration of trabecular network post chemo-induced injury remains attenuated by co-treatment with the Notch $\gamma$-secretase inhibitor DAPT at 7 dpf. Treatment with Notch downstream effectors NICD and NRG1 rescues trabecular myocardium. ANOVA and Tukey’s method, * $P<0.05$, ** $P<0.01$, n=6-8 per group.
Supplemental Figure S5. Contractile function of *ex vivo* adult zebrafish cardiomyocytes following doxorubicin treatment. (A-B) Relaxed cardiomyocyte with length $L_1$. (C-D) Contracted cardiomyocyte with length $L_2$. (E) The percentage cell shortening of cardiomyocytes decreased significantly following doxorubicin treatment, and co-treatment of doxorubicin with DAPT. Overexpression of $NRG1$ rescued the reduction of contractility. * $P<0.05$, ** $P<0.01$, n=7-10 per group. Scale bar: 30 μm.
Supplemental Figure S6. Inhibition of Notch signaling by DAPT at 0, 24, and 48 hours post doxorubicin-induced injury leads to differential restoration of segmental cardiac function. (A) Doxorubicin was combined with DAPT treatment at various time-points. (B) Synchronous co-treatment of doxorubicin with DAPT impaired the restoration of myocardial displacement in both segment I and VI. However, Notch inhibition by DAPT at 24 and 48 hours post doxorubicin injury impaired the restoration of segmental cardiac function only in segment I. * P<0.05, n=6 per group.
Supplemental Figure S7. DIAMOND mechanics at 7 dpf for segmental localization and quantification of NICD and NRG1 mRNA-mediated myocardial protection following doxorubicin-induced injury. The inhibition of Notch signaling by the γ-secretase-inhibitor DAPT impaired the restoration of segmental (A, DIAMOND) and global (B, ejection fraction) cardiac function, a finding that was rescued NICD and NRG1 mRNA treatment. ANOVA and Tukey’s method, †† P<0.01 Dox + DAPT vs. control, * P<0.05, ** P<0.01, n=6-10 per group.
Supplemental Table S1. Characteristics of imaging modalities. A semi-quantitative scale ranging from + to +++ was used to compare key characteristics between imaging modalities.

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<th>NICD mRNA sequence.</th>
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Supplemental Table S3. *NRG1* mRNA sequence.

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CTTCTACAGTACGTCCACTCCCTTCTGTCTGCTGCTGACTAG
SUPPLEMENTAL VIDEOS

Supplemental Video S1. 4-D cardiac cycle with DIAMOND and raw data.

Supplemental Video S2. 4-D and time-dependent 2-D intracardiac views in control fish at 4 dpf.

Supplemental Video S3. 4-D and time-dependent 2-D intracardiac views in doxorubicin treated fish at 4 dpf.

Supplemental Video S4. 4-D and time-dependent 2-D intracardiac views in control fish at 6 dpf.

Supplemental Video S5. 4-D and time-dependent 2-D intracardiac views in doxorubicin treated fish at 4 dpf.